Kwizera et al. World Allergy Organization Journal (2020) 13:100130
http://doi.org/10.1016/j.waojou.2020.100130

WORLD ALLERGY ORGANIZATION JOURNAL

Open Access

Skin prick reactivity among asthmatics in East Africa

Richard Kwizera[a,b]*, Vincent Wadda[c], Levicatus Mugenyi[b], Hellen Aanyu-tukamuhebwa[b,d], George Nyale[e], Getnet Yimer[f], Jeremiah Chakaya[g], Corina De jong[h], Thys Van der molen[h], David W. Denning[i], Robin Gore[j] and Bruce J. Kirenga[b,c,d]**

ABSTRACT

Background: The burden of asthma in Africa is high, and yet the disease is not universally prioritised. Data on allergic asthma and its impact on asthma morbidity are limited in Africa. Our aim was to describe the distribution of skin prick positivity among asthmatics in Eastern Africa.

Methods: From August 2016 to May 2018, 1671 asthmatic patients were enrolled from Uganda, Kenya, and Ethiopia as part of the African Severe Asthma Program clinical study. Skin prick testing was performed at baseline using a panel of 12 allergens, and factors associated with skin prick reactivity determined.

Results: Of the 1, 671 patients recruited, 71% were female with a median age of 40 years, 93.6% were aged >15 years and the patterns of asthma symptom frequency was intermittent in 2.9%, mild persistent in 19.9%, moderate persistent in 42.6% and severe persistent in 34.6% at baseline. Self-reported triggers, were dust (92%), cold weather (89%), upper respiratory infections (84%), strong smells (79%) and exposure to tobacco (78%). The majority (90%) of the participants had at least 1 positive allergen reaction, with 0.9% participants reacting to all the 12 allergens. Participants commonly reacted to house dust mites (66%), *Blomia tropicalis* (62%), and the German cockroach (52%). Patients sensitized to more allergens (>2) had significantly reduced lung function (FEV $\leq$ 80%; p = 0.001) and were more likely to visit the emergency department due to asthma (p = 0.012). There was no significant relationship between number of allergens and measures of asthma control, quality of life, and other clinical outcomes. Only the country of origin was independently associated with atopy among African asthmatics.

Conclusion: There is a high prevalence of skin prick positivity among East African patients with asthma, with the commonest allergen being house dust mite. Skin reactivity did not correlate well with asthma severity and poor asthma control. The relation between atopy, measured through skin prick testing, and measures of asthma control among asthma patients in Eastern Africa is unclear and needs further study.

[a]Translational Research Laboratry, Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda
*Corresponding author. c, MSc, MSc (UK), Laboratory Manager, Translational Research Laboratory, Department of Research, Infectious Diseases Institute-Mulago, College of Health Sciences, Makerere University P.O. Box 22418, Kampala, Uganda. E-mails: rkwizera@idi.co.ug; kwizerarichard@ymail.com
**Corresponding author. Makerere University Lung Institute, College of Health Sciences, Makerere University, Kampala, Uganda.

Full list of author information is available at the end of the article

http://doi.org/10.1016/j.waojou.2020.100130
Received 22 October 2019; Received in revised from 6 May 2020; Accepted 14 May 2020
Online publication date xxx
1939-4551/

Trial registration: The ASAP study was registered prospectively. ClinicalTrials.gov Identifier: NCT03065920; Registration date: February 28, 2017; Last verified: February 28, 2017.

Keywords: Allergy, Atopy, Asthma, SPT, East Africa, Africa

BACKGROUND

Asthma is a chronic lung disorder affecting the airways, with a complex pathophysiology that involves airway inflammation, intermittent airflow obstruction, and bronchial hyper-responsiveness.[1–3] The prevalence of asthma in Africa is estimated to be between 6 and 12%,[4–6] with national estimates ranging from 2 to 53%.[4] Symptoms of asthma may be increased or worsened by different aeroallergens that the patients encounter.[7] More than 50% of adult asthma and 80% of childhood asthma has been reported to be allergic; there is evidence that the prevalence of allergic asthma is increasing.[8] The allergens act locally in the airways in a classical type-I hypersensitivity reaction, leading to bronchospasm and type-2 allergic inflammation.[2,3] Therefore, early allergy testing could be beneficial in persistent and intermittent asthma particularly for indoor aeroallergens.

Skin prick testing (SPT) is currently the most effective diagnostic test to detect immunoglobulin (Ig) E mediated type-I hypersensitivity reactions especially in patients with atopic asthma.[9,10] SPT is considered simple, safe and highly sensitive, with low cost and reproducible results.[11,12] Measurement of allergen specific IgE in serum has been shown to have little advantage over properly performed SPT.[13] SPT has also been shown to be more sensitive than radioallergosorbent tests for detection of IgE reactivity.[14] SPT reactions have also been shown to be associated with patient-reported clinical symptoms.[15]

However, most of this evidence base is from high income settings and few studies have been carried out in Africa[16,17] to explore the distribution of allergic asthma. The aim of this study was to describe the allergen sensitivities (atopy) and factors associated with it among asthmatics enrolled in the African Severe Asthma Project (ASAP), a multi-national clinical study conducted in the Eastern Africa.

MATERIALS AND METHODS

Study design

This was a nested cross-sectional study under the African Severe Asthma Program (ASAP) clinical study (*ClinicalTrials.gov Identifier:* NCT03065920).[18,19] ASAP was a prospective clinical study with the primary objective of identifying and characterizing severe asthma in Eastern Africa, in order to understand its demographic, clinical, physiologic, pathologic, genomic, and immunologic determinants. ASAP was a multi-site study conducted at: Makerere University College of Health Sciences at Mulago Hospital in Uganda, Kenyatta National Hospital in Nairobi, Kenya, and Black Lion Hospital, Addis Ababa College of Health Sciences in Ethiopia.

Study population and inclusion criteria

The study included asthmatics aged 12–70 years, residing within 30 km of the enrolling sites. Patients with a current/previous doctor diagnosis of asthma or clinical/treated asthma or wheezing/whistling breath in the last 12 months were eligible for enrolments into the study. We excluded patients with an alternative lung disease (e.g. COPD), comorbid diseases likely to confound assessment of asthma (eg, active TB), patients unable to perform study tests and procedures and pregnant women.

Study procedures

In patients with a history suggestive of asthma in the last 12 months, asthma was diagnosed using 2 criteria: clinical diagnosis of asthma by a primary physician (doctor-diagnosed asthma) and a spirometric lung function test that confirmed presence of airflow obstruction. After giving informed consent, patients were enrolled and underwent a

respiratory focused clinical evaluation using a pre-developed clinical review form to collect data on demographics, asthma symptoms, asthma control, exposure to outdoor and indoor pollutants, known asthma triggers, tobacco smoking, vital signs, respiratory system physical signs, hospitalisation, adverse events, and visit to the emergency department. Detailed procedures for tests such as lung function tests, stool examinations, and blood tests were published by Kirenga et al.[19] Asthma control was assessed at each visit using the asthma control test (ACT).[20] In the ACT, good asthma control was defined as having none of the following in the last 4 weeks: "night-time asthma symptoms, asthma symptoms on waking, need for reliever medication, restriction of day-to-day activities, days off school or work due to asthma, and asthma attacks or flare-ups". ACT was categorized into a binary variable where "controlled" was defined as ACT score of ≥ 20 and "uncontrolled" being ACT score <20. Asthma severity was assessed using the definitions and diagnostic criteria provided by the WHO.[21] The Asthma Quality of Life Questionnaire (AQLQ) was used to assess the quality of life of the asthma patients.[22] Blood was collected and tested for HIV and eosinophil count. Stool was collected and tested for parasitic infections. Lung function tests were also performed.

Skin prick test procedures

Skin Prick Tests (Immunospec [Pty] Ltd, Johannesburg, Gauteng, South Africa) were performed at baseline for all patients enrolled into the ASAP study. SPT were performed and interpreted according to published international guidelines.[23,24] The procedure was performed in a special clinic room by a trained team. Allergens included the following; *Aspergillus fumigatus*, Mould mix IV (*Penicillium brevicompactum, Penicillium expansum, Penicillium notatum*), house dust mite mix, soya bean, *Blomia tropicalis* (tropical house dust mite), Bermuda grass, dog epithelia, cat epithelia, German cockroach, egg white, cow milk, and peanut. These are the presumed commonest allergens to which patients are exposed in the African region. Normal saline served as a negative control while histamine was the positive control, with a mean wheal diameter of at least 3 mm being positive test read after 15 min of allergen application.[25] Wheal size was recorded on the SPT form. Although the risk for systemic reactions in SPT is very small,[26] necessary emergency measures were ensured to deal with any systemic allergic reaction or anaphylaxis.

Quality control for the SPT

The study staff were trained in the SPT procedures and training logs maintained prior to study initiation as described before.[27] The study staff took a comprehensive patient's history to ensure that the patients had not been taking antihistamines or oral steroids in the last 7 days prior to the SPT. A second reader was called upon for reactions that were not clear. The allergen reagents were kept in a refrigerator at 2–8 °C when not in use. The study quality control officer reviewed the SPT forms and checked them for completeness and accuracy.

Statistical analysis

Data were analysed using STATA® version 14 (STATA, College Station, Texas). Primary data analysis aimed to describe the distribution of skin prick positivity and to determine factors associated with it among studied asthmatics at a 95% confidence interval. The relationship between SPT reaction and asthma severity/control was also assessed.

RESULTS

Characteristics of the study population

Between August 2016 and May 2018, 2242 patients were screened and 1671 consenting patients who met the inclusion criteria were recruited into the study with the following study site distribution: Uganda 821 patients; Kenya 431 and Ethiopia 419 patients. Because the study sites were in urban areas and recruitment of patients into the study limited to those who reside within 30 km of the study sites, enrolled patients were mostly urban residents (>90%). Therefore, data were not available to evaluate asthma and allergic sensitisation in rural vs urban settings. Overall, 71% (1170/1660) of the participants were female, the whole group had an overall median age of 40 years (IQR; 26 to 52, $n = 1634$). The majority of the participants (20%) were in the age group of 35–44 years. The

overall median age at first diagnosis of asthma was 25 years (IQR; 14 to 36, n = 1623). Similarly, most of our participants were in their young and most productive age, but with the largest percentage having moderate and severe persistent asthma. Among patients with asthma status recorded at baseline (n = 1649), 2.9% had intermittent, 19.9% had mild persistent, 42.6% had moderate persistent while 34.6% had severe persistent asthma. Only 1606/1671 participant's performed spirometry, based on this 66.3% were diagnosed as having confirmed asthma. Of the 1671 patients, baseline assessment revealed the following comorbidities: rhinosinusitis in 21% (352/1671), eczema/dermatitis in 7% % (112/1671), while 4% (63/1671) were infected with HIV infection. Only 0.2% (3/1667) were current smokers, 7% (110/1667) were former smokers, while the majority (93% [1554/1667]) had never smoked. The majority (73% [1221/1664]) of the participants were exposed to biomass smoke while only 20% used kerosene for lighting or cooking (Table 1a). In Table 1b, we present the baseline characteristics of only those participants (93%) who had full datasets with all parameters; and the results were not so different from when everyone was included in Table 1a.

Self-reported triggers of asthma symptoms

The commonest self-reported triggers of asthma symptoms among patients recruited in our study population (n = 1671) were dust (92%), cold weather (89%), upper respiratory infections (84%), strong smells (79%), exposure to tobacco (78%), and strong emotions (50%) Among participants in whom skin prick test was done (n = 1287), 90% (1160/1287) had at least 1 self-reported trigger. Among the 5 patients who had no self-reported triggers, only the 3 from Kenya had at least 1 allergen positive by skin prick test. The 2 Ethiopians who had no self-reported triggers did not have any reactions with skin prick test.) (Table 2).

Allergen distribution

Overall, 90.4% (1163/1287) of the participants had at least 1 allergen positive using skin prick test, with positivity rates of 96% (695/721) in Uganda, 78% (285/364) in Kenya and 91% (183/202) in Ethiopia. The overall positivity rate for each allergen was; house dust mite mix (66%), *Blomia tropicalis* (62%), German cockroach (52%), *Aspergillus fumigatus* (32%), Bermuda grass (31%), mould mix IV (29%), cat epithelia (28%), dog epithelia (23%), cow milk (22%), peanut (22%), egg white (19%), and soya bean (18%). Rates for each allergen varied widely by country. Fig. 1 and Fig. 2 show the percentage positivity for each allergen per country for adults and children respectively (Figs. 1 and 2).

The number of allergens that each participant reacted to per country were explored. Ten per cent (124/1287) of the participants did not react to any of the above allergens included in the panel, while 0.9% (11/1287) reacted to all the 12 allergens included in the SPT panel. Twenty eight percent of the participants reacted to more than 5 allergens. The number of positive allergen reactions per patient also varied widely across the 3 countries (Fig. 3).

Effect of allergens on asthma morbidity

Ethiopia registered the highest proportion of severe persistent asthmatics (58.37%) followed by Kenya (37.03%) and least in Uganda (21.61%). The association between individual allergens and asthma severity/status and asthma control was assessed in this cohort. In bivariate analysis, no individual allergen sensitisation was significantly associated with asthma status/severity at baseline. However, in Uganda, mould mix sensitisation was found significantly more often in severe asthmatics ($p < 0.0001$). In addition, the proportions of *A. fumigatus* ($p = 0.001$), house dust mite ($p = 0.001$), German cockroach ($p = 0.035$) and cow milk sensitisation were significantly different by asthma status with more positives among intermittent asthmatics in Uganda. In Kenya, cow milk sensitisation was significantly more common among moderate asthmatics ($p = 0.05$). In Ethiopia, sensitisation to *A. fumigatus* ($p = 0.014$), mould mix ($p = 0.042$) and house dust mites ($p = 0.043$) was significantly more frequent among moderate asthmatics.

Similarly, in bivariate analysis across the 3 countries, with reference to asthma control (binary variable; controlled vs uncontrolled) Bermuda grass ($p = 0.049$), egg white ($p = 0.006$) and cow milk ($p = 0.018$) sensitisation was significantly more common among the controlled asthmatics. In Uganda, none of the allergens were significantly

Characteristics	Overall (N = 1671)	Uganda (N = 821)	Kenya (N =431)	Ethiopia (N =419)
Gender	N = 1660	N = 815	N = 431	N = 414
Female (%)	1170 (70.5)	609 (74.7)	322 (74.7)	239 (57.7)
Age (years)	**N = 1634**	**N = 810**	**N = 430**	**N = 394**
Median age (IQR)	40 (26 - 52)	31 (20 - 44)	42 (32 - 51)	52 (42 - 60)
Age categories (years) n (%)	**N = 1634**	**N = 810**	**N = 430**	**N = 394**
12-14	105 (6.4)	81 (10.0)	19 (4.4)	5 (1.3)
15-24	271 (16.6)	211 (26.1)	48 (11.2)	12 (3.1)
25-34	260 (15.9)	167 (20.6)	65 (15.1)	28 (7.1)
35-44	329 (20.1)	154 (19.0)	105 (24.4)	70 (17.8)
45-54	320 (19.6)	99 (12.2)	116 (27.0)	105 (26.7)
55-64	233 (14.3)	76 (9.4)	48 (11.2)	109 (27.7)
65+	116 (7.1)	22 (2.7)	29 (6.7)	65 (16.5)
Age at first diagnosis of asthma	**N = 1623**	**N = 813**	**N = 400**	**N = 413**
Median age (IQR)	25 (14 - 36)	20 (10 - 33)	29 (16 - 39)	29 (22 - 36)
Asthma status, n (%)	**N = 1649**	**N = 819**	**N = 424**	**N = 406**
Intermittent	47(2.9)	16(1.9)	30(7.1)	1(0.3)
Mild persistent	328(19.9)	215(26.3)	78(18.4)	35(8.6)
Moderate persistent	703(42.6)	411(50.2)	159(37.5)	133(32.8)
Severe persistent	571(34.6)	177(21.6)	157(37.0)	237(58.4)
Suffering/ diagnosed with; n (%)	**N = 1671**	**N = 821**	**N = 431**	**N = 419**
Rhino sinusitis	352 (21.1)	247 (30.1)	88 (20.4)	17 (4.1)
Eczema/dermatitis	112 (6.7)	61 (7.4)	34 (7.9)	17 (4.1)
HIV	63 (3.8)	48 (5.9)	9 (2.1)	6 (1.4)
History of smoking n (%)	**N = 1667**	**N = 821**	**N = 430**	**N = 416**
Current smoker	3 (0.2)	1 (0.1)	1 (0.2)	1 (0.2)
Former smoker	110 (6.6)	38 (4.6)	37 (8.6)	35 (8.4)
Never	1554 (93.2)	782 (95.3)	392 (91.2)	380 (91.4)
Exposed to biomass smoke n (%)	**N = 1664**	**N = 821**	**N = 428**	**N = 415**
Yes	1221 (73.4)	643 (78.3)	274 (64.0)	304 (73.3)
Uses kerosene for lighting or cooking n (%)	**N = 1667**	**N = 821**	**N = 430**	**N = 416**
Yes	334 (20.0)	113 (13.8)	144 (33.5)	77 (18.5)

Table 1a. Baseline characteristics of study population for all participants by country. *Data presented are n (%) or medians with interquartile ranges (IQR). N = number of participants with data for each variable*

Characteristics	Overall (N = 1554)	Uganda (N = 795)	Kenya (N =392)	Ethiopia (N =367)
Gender: Female, n (%)	1106 (71.2)	596 (75.0)	294 (75.0)	216 (58.9)
Age (years), median (IQR)	40 (26-52)	31 (20-44)	42 (32-52)	52 (42-60)
Age categories (years), n (%)				
12-14	100 (6.4)	79 (9.9)	16 (4.1)	5 (1.4)
15-24	255 (16.4)	207 (26.0)	38 (9.7)	10 (2.7)
25-34	253 (16.3)	163 (20.5)	63 (16.1)	27 (7.4)
35-44	310 (20.0)	151 (19.0)	93 (23.7)	66 (18.0)
45-54	309 (19.9)	99 (12.5)	110 (28.1)	100 (27.3)
55-64	219 (14.1)	74 (9.3)	44 (11.2)	101 (27.5)
65+	108 (7.0)	22 (2.8)	28 (7.1)	58 (15.8)
Age at first diagnosis of asthma, Median (IQR)	25 (14-36)	20 (10-33)	29 (16-39.5)	28 (22-35)
Asthma status, n (%)				
Intermittent	44 (2.8)	16 (2.0)	27 (6.9)	1 (0.3)
Mild persistent	315 (20.3)	209 (26.3)	75 (19.1)	31 (8.5)
Moderate persistent	667 (42.9)	399 (50.2)	145 (37.0)	123 (33.5)
Severe persistent	528 (34.0)	171 (21.5)	145 (37.0)	212 (57.8)
Suffering/ diagnosed with, n (%)				
Rhino sinusitis	330 (21.2)	240 (30.2)	75 (19.1)	15 (4.1)
Eczema/dermatitis	103 (6.6)	59 (7.4)	28 (7.1)	16 (4.4)
HIV	63 (4.1)	48 (6.0)	9 (2.0)	6 (1.6)
History of smoking, n (%)				
Current smoker	3 (0.2)	1 (0.1)	1 (0.3)	1 (0.2)
Former smoker	99 (6.4)	37 (4.7)	33 (8.4)	29 (7.9)
Never	1452 (93.4)	757 (95.2)	358 (91.3)	337 (91.8)
Exposed to biomass smoke, n (%)				
Yes	1143 (73.6)	622 (78.2)	251 (64.0)	270 (73.4)
Uses kerosene for lighting or cooking, n (%)				
Yes	319 (20.5)	108 (13.6)	139 (35.5)	72 (19.6)

Table 1b. Baseline characteristics of participants with complete datasets by country. *Data presented are n (%) or medians with interquartile range (IQR). N = number of participants with complete data for all variables*

	Overall n (%) N = 1671	Uganda n (%) N = 821	Kenya n (%) N = 431	Ethiopia n (%) N = 419
Upper respiratory infections	1407 (84.2)	811 (98.8)	252 (58.5)	344 (82.1)
Exposure to household pets/poultry	156 (9.3)	67 (8.2)	75 (17.4)	14 (3.3)
Smoking/exposure to tobacco	1296 (77.6)	771 (94.5)	340 (78.9)	180 (43.0)
Strong emotions	832 (49.8)	500 (60.9)	142 (33.0)	190 (45.4)
Cold weather	1481 (88.6)	725 (88.3)	387 (89.8)	369 (88.1)
Hypertension drugs or aspirin	28 (1.7)	10 (1.2)	15 (3.5)	3 (0.7)
Exercise	723 (43.3)	342 (41.7)	132 (30.6)	249 (59.4)
Dust	1540 (92.1)	789 (96.1)	388 (90.0)	363 (86.6)
Strong smells or perfumes	1323 (79.2)	686 (83.6)	344 (79.8)	293 (69.9)
Exposure at work	56 (3.4)	25 (3.1)	21 (4.9)	10 (2.4)
Other	15 (0.9)	14 (1.7)	0 (0.0)	1 (0.2)
None of the above	5 (0.3)	0 (0.0)	3 (0.7)	2 (0.5)

Table 2. Self-reported triggers by country. *Data presented are n (%). N = number of participants with data for each parameter*

associated with asthma control. In Kenya, German cockroach sensitisation was found significantly more often among the uncontrolled asthmatics (p = 0.003) while cow milk sensitisation was more frequent among controlled asthmatics (p = 0.018). In Ethiopia, the proportions of those with *A. fumigatus* (p = 0.014), *Blomia tropicalis* (p = 0.029) and dog epithelia (p = 0.031)

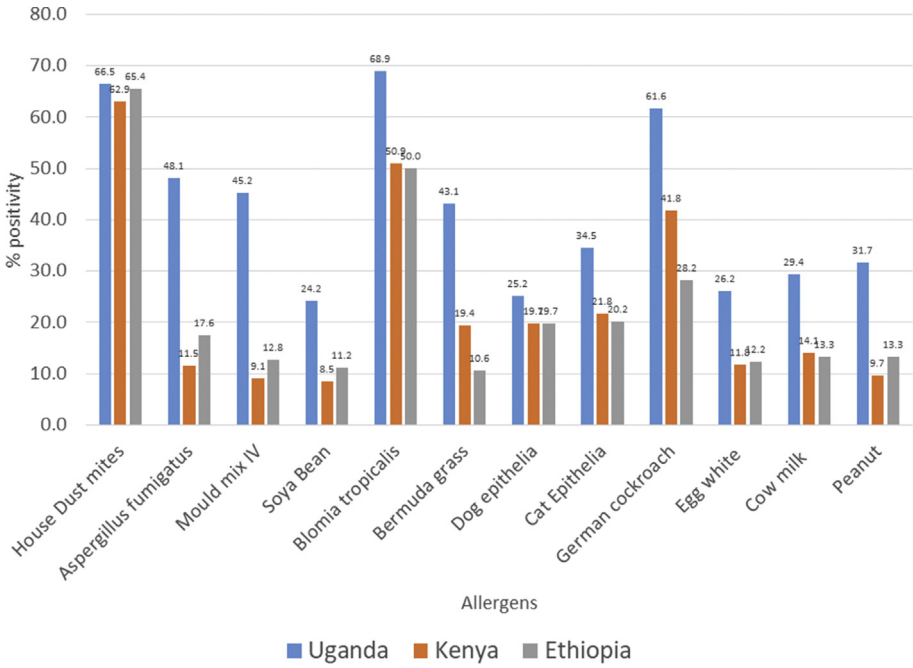

Fig. 1 Distribution of allergens in adults. Figure shows the percentage positivity for each allergen per country among adults (16+ years). Participants mostly reacted to house dust mites, *Blomia tropicalis* and the German cockroach

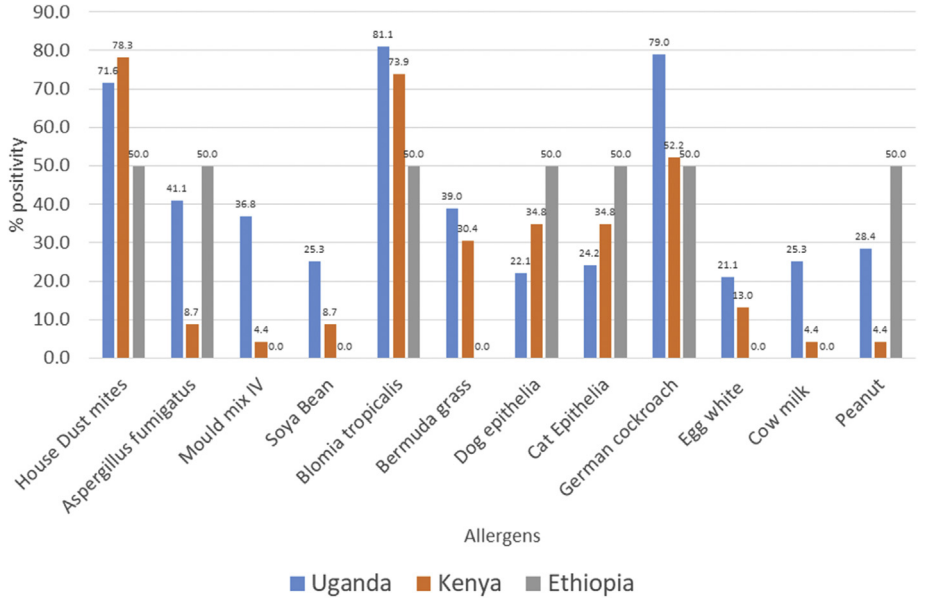

Fig. 2 Distribution of allergens in children. Figure shows the percentage positivity for each allergen per country among adults (12–15 years). Participants mostly reacted to house dust mites, *Blomia tropicalis* and the German cockroach

sensitisation were more often positives among the controlled asthmatics.

There was a strong association between the number of allergens to which a patient was sensitized and the lung function. Patients sensitized to more allergens had significantly lower lung function (FEV$\leq$ 80%; p = 0.001). Similarly, patients sensitized to more allergens were more likely to visit the emergency department due to asthma (p = 0.012). There was no significant relationship between number of allergens and asthma control, quality of life, and other clinical outcomes.

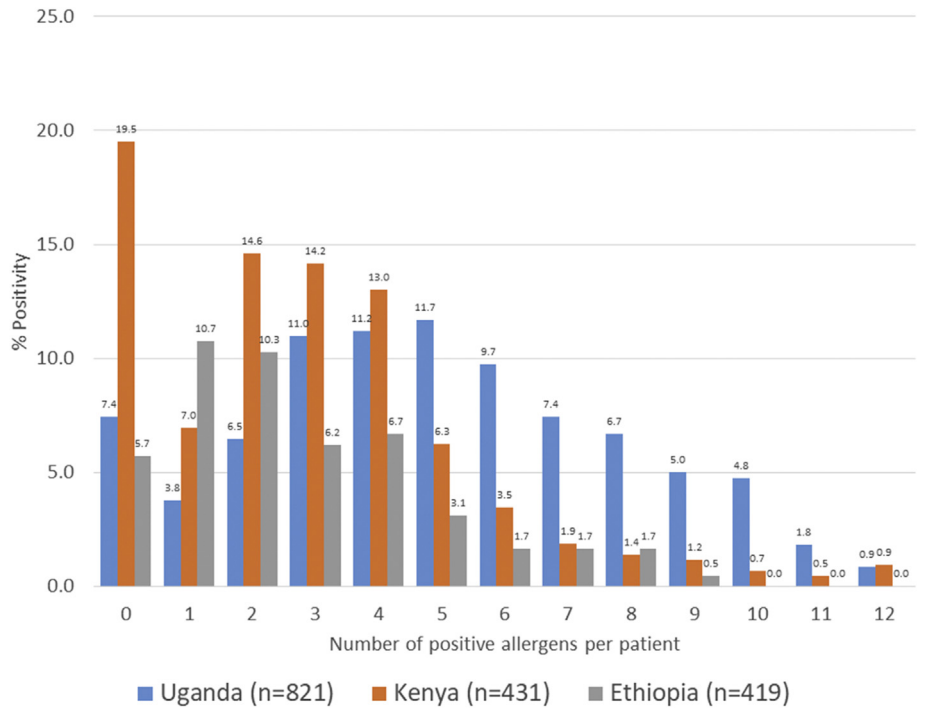

Fig. 3 Percentage positivity of allergens per patient. The number of positive allergens per patient varied widely across the 3 countries. Ten percent of the participants did not react to any of the allergens, while 1% reacted to all the 12 allergens. Ugandans had a noticeably higher degree of atopy

Characteristic	CRUDE			ADJUSTED		
	OR	95% CI	P value	OR	95% CI	P value
country of origin						
Kenya	0.14	0.09–0.22	<0.001	0.09	0.04–0.24	<0.001
Ethiopia	0.36	0.19–0.67	0.001	0.23	0.08–0.66	0.006
Exposure to cockroaches	1.58	1.08–2.29	0.017	0.48	0.22–1.01	0.053
Visit to casualty clinic because of asthma	1.68	1.19–2.38	0.003	1.03	0.64–1.65	0.915
Attacks/exacerbations	1.25	1.02–1.52	0.028	1.07	0.71–1.60	0.751
Course of oral steroids prescribed	1.43	1.08–1.89	0.011	1.41	0.88–2.24	0.154
Age	0.99	0.98–0.99	0.02			
Sex: Female	0.78	0.50–1.21	0.259			
Clinic visit because of asthma	1.3	1.03–1.06	0.031			
Upper respiratory infection	2.21	1.44–3.39	<0.001			
High blood pressure drugs and pain killers like aspirin	0.35	0.13–0.97	0.044			
Exposure at work	0.45	0.20–0.99	0.048			

Table 3. Factors associated with allergy among asthmatics in Africa, with the country reference being Uganda. *Data presented are odds ratios (OR), 95% confidence intervals (CI) and P-values. Allergy was defined as presence of sensitisation with a SPT of ≥ 3 mm to at least 1 allergen*

Factors associated with allergy among African asthmatics

A crude logistic regression analysis was performed to establish factors associated with atopy among our cohort of asthmatics. The following parameters were shown to be significantly associated with atopy among our cohort of asthmatics in the crude analysis; country of origin, exposure to cockroaches, visit to emergency clinic because of asthma, attacks/exacerbations, courses of oral steroids prescribed, age, visit to a clinic because of asthma, upper respiratory infections, exposure at work, and high blood pressure drugs/pain killers like aspirin. However, in the adjusted analysis using a model, only country of origin was independently associated with allergy among our cohort of asthmatics (Table 3).

DISCUSSION

We prospectively demonstrated a high prevalence (90%) of atopy (ie, skin prick positivity) in this Eastern Africa cohort of asthmatic population across 3 countries. Dust was the most prevalent self-reported trigger. The participants in this cohort mostly reacted to house dust mites, the tropical mite *Blomia tropicalis* and the German cockroach. Patients sensitized to more allergens had significantly reduced lung function and were more likely to visit the emergency department due to asthma.

Ugandan asthmatics had the highest levels of reactions for all the allergens followed by Kenyans and the least reactions were recorded in Ethiopia. Only country of origin was independently associated with allergy among our cohort of asthmatics. Some allergens were also significantly associated with severity at baseline. More studies are needed to explore allergic asthma in Africa.

The high prevalence (90%) of skin prick reactivity observed in this study was similar to what has been reported before by other studies in different countries. A recent study done in Uganda to estimate the prevalence of atopic sensitisation among women, revealed a 70% (14/20) skin reactivity among asthmatics and 32% (37/117) among controls.[28] A similar study done among Malaysian asthmatic patients with and without rhinitis showed that 68% were reactive to at least 1 of the aeroallergens.[29] An earlier case control study carried out among 576 asthmatics and 144 healthy controls in Puerto Rico showed that 86% of the asthmatics had at least 1 positive skin reaction.[30] A similar study done in Jeddah, Saudi Arabia among 151 asthma patients gave a 75% skin reactivity to at least 1 allergen; but without a control group.[31] However, in the current study, there was no clear explanation to why Uganda had the highest rates of sensitisation. This might be related to the climate, pollution, humidity, and other metrological factors that are specific to each country.

The asthmatics enrolled in this study mostly reacted to house dust mite, *Blomia tropicalis* and the German cockroach. House dust allergens were earlier shown to be associated with childhood atopic asthma.[32,33] Over 50% of children sensitized to house dust mite have asthma.[34] This observation may be similar in adults.[35] A study done in Brazil among asthmatic children similarly showed a high reactivity to both *Dermatophagoides pteronyssinus* and *B. tropicalis* and recommended environmental control of house dust mite exposure. In this study, *Dermatophagoides farinae* was found in very low levels (<0.5 μg/g).[36] A dust-free bedroom was considered as 1 of the practical and effective methods for decreasing asthma in children with house dust mite allergy.[37] However, recent evidence shows that this method is clinically ineffective in adults with asthma.[38] Sensitivity to house dust mite has a very strong independent association with current asthma, but not asthma severity.[39] However, recent evidence shows that exposure to house dust mite allergens is not associated with asthma in childhood.[40] In fact, the chemical and physical methods used to reduce exposure to house dust mite allergens are not recommendable.[41] This sensitisation against house dust mites may translate into increased prevalence of allergic airway diseases.[42]

Similarly, the German cockroach was associated with severe persistent asthma at base line in Kenya. Cockroaches are known to produce many proteins that induce IgE antibody responses with a high reactivity using skin prick test and potential cross-reactivity with mite allergens.[43] German cockroach feces have also been shown to be rich in allergens.[44] Exposure and sensitisation to these cockroach allergens is associated with increased asthma morbidity especially among African American and Hispanic populations.[45]

In the current study, we registered a relatively high prevalence of skin reactivity to *Aspergillus fumigatus* (32%) and the mould mix IV (29%). Mould mix was the only allergen that was associated with the characteristic of severe persistent asthma at baseline, but only in Uganda. Fungal allergy has been known to be associated with worse asthma control, leading to asthma attacks,[46] additional need for corticosteroids, hospitalisation[47] and increased rates of bronchiectasis (especially due to *A. fumigatus*).[48–50] However, other studies too have demonstrated a weak association between *Aspergillus* sensitisation and asthma severity.[51] The skin prick test panel used in the current study did not include other relevant fungal allergens such as *Alternaria* and *Cladosporium* species. The mould mix too was composed of 3 *Penicillium* species only (*P. brevicompactum, P. expansum,P. notatum*). Besides, the index of clinical suspicion for fungal infections is very low in Uganda.[52,53] A recent review investigating the burden of fungal asthma in Africa showed that the prevalence of fungal allergy was high (3–52%) in adult asthmatics in Africa with an average of 28% and a pooled estimate of 23%. This was mostly caused by *Aspergillus* species, followed by *Alternaria* species (6–40%), *Cladosporium* species (4–42%) and the mould mix (7–11%).[6] The review

estimated that approximately 4 million adult asthmatics have fungal sensitisation in Africa. For future studies, *Alternaria* species and *Cladosporium* species need to be investigated in the African population.

Dust was the commonest self-reported trigger in this cohort across the 3 countries. This was probably because majority of the participants resided in the busy urban areas where the levels of dust pollution are highest. Urbanisation and air pollution with dust and other substances have been implicated in the increasing burden of asthma in Africa.[4,54]

Surprisingly, food allergies were significantly associated with mild forms of asthma at baseline. In addition, patients sensitized to more allergens had significantly reduced lung function and were more likely to visit the emergency department due to asthma. Therefore, with a potential increase in the disability-adjusted life years due to allergic asthma, this is expected to have serious negative impact on the developing economies for the different African countries.

Over 70% of the participants were female and there was no significant relationship between gender and allergy. This difference could have introduced bias because of differences in cigarette or other noxious agent exposures, although >90% of participants had never smoked. The median age at first diagnosis of asthma of 25 years was relatively high; which may be the result of late presentation and/or poor health seeking habits rather than late on-set. It is significant to note that we did not probe specifically for time of onset of symptoms which is among the limitations of this study. The poor health seeking habits in Africa are especially seen among male participants.[55] Previous studies have shown mean age at first diagnosis of asthma to range from 2.6 to 16 years,[56–59] which is lower than the 25 years in our cohort.

Unbiased cohort studies indicate that asthma can be thought of as a series of disease clusters, in which atopy is variably represented. Incompletely understood polygenetic determinants no doubt influence the expression of atopy while clinical asthma severity is thought to occur through the interplay of genetics, microbial exposures, and multiple environmental factors, which include but are not limited to aeroallergens and pollutants.[60–62] Similarly, the median eosinophil counts were normal across the 3 countries and 10% of our participants did not react to any of the allergens included in the panel. Stool tests were performed to check parasitic infections, and all these were negative for all participants. It is still unclear why some patients have allergic asthma and others do not, although there is an apparent genetic vulnerability.[63] In this case, interventions that decrease vulnerability to air pollution in genetically susceptible individuals may have a role in personalized asthma management. Other allergic comorbidities like rhinosinusitis (21%) and eczema/dermatitis (7%) were also registered in this population. However, previous studies have shown that rhinosinusitis occurs in 75–90% of allergic asthmatics and 80% of those with non-allergic asthma.[64]

The main limitation to the study is that the results presented in this study were from 3 East African countries and the conclusions may not be applicable to all African countries or even the entire country in which the study was undertaken – because this was a single center study in each country. In this paper we only present base line data pending determination of the relationship between atopic sensitisation and response to optimized treatment. The diagnosis of severe asthma at baseline was based on symptom and clinical events recall which may have introduced biases. There is a significant difference between the percentage of male and female population, and this may be a bias for risk factors. The majority (>90%) of our participants resided in the urban areas of their respective countries and thus there is still an information lacuna about skin prick reactivity and asthma characteristics between urban and rural populations.

CONCLUSION

There is a high prevalence of skin prick positivity among East African patients with asthma with the commonest allergen being house dust mite. Skin reactivity did not correlate well with asthma severity and poor asthma control. The relation between atopy, measured through skin prick testing, and measures of asthma control among

asthma patients in Eastern Africa is unclear and needs further study.

Abbreviations

FEV: Forced Expiratory Volume; ASAP: African Severe Asthma Project; SPT: Skin prick testing; Ig: Immunoglobulin; COPD: Chronic obstructive pulmonary disease; TB: Tuberculosis; ACT: Asthma control test; AQLQ: Asthma Quality of Life Questionnaire; HIV: Human immunodeficiency virus; IQR: Interquartile range; A. fumigatus: Aspergillus fumigatus

Potential competing interests

All authors report no competing interests.

ETHICAL CONSIDERATION

ASAP study obtained ethical approval in Uganda and all the three partner sites. Participants provided written informed consent to participate in the ASAP study. Ethics approval for this sub-study was received from the Mulago Hospital Research and Ethics committee and the Uganda National Council for Science and Technology and from ethics committees in each country.

Abbreviations

FEV: Forced Expiratory Volume; ASAP: African Severe Asthma Project; SPT: Skin prick testing; Ig: Immunoglobulin; COPD: Chronic obstructive pulmonary disease; TB: Tuberculosis; ACT: Asthma control test; AQLQ: Asthma Quality of Life Questionnaire; HIV: Human immunodeficiency virus; IQR: Interquartile range; A. fumigatus: Aspergillus fumigatus

Authors' contributions

BJK conceived and designed concept. RK, VW, GN, GY performed experiments. RK, LM analysed data. RK participated in initial manuscript drafting. RK, VW, LM, HAT, GN, GY, JC, CDJ, TVDM, DWD, RG, BJK participated in critical revisions for intellectual content. BJK, TVDM participated in obtaining funding. BJK, CDJ, GY, JC participated in administrative, technical, or material support.

Funding

ASAP was funded by the GlaxoSmithKline African Non-Communicable Disease (NCD) Open Lab grant # 3000030409 to BJK. The funders had no role in study design; collection, analysis and interpretation of data, in the writing of the report; and in the decision to submit the article for publication.

Consent for publication

Written consent was obtained from all the participants to publish the anonymised details.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files. The authors confirm that all data underlying the findings are fully available without restriction and can be availed by contacting Mr. Richard Kwizera (kwizerarichard@ymail.com).

Acknowledgements

We are grateful for institutional support from the Makerere University Lung Institute. We also thank the entire ASAP team for patients' care. RK is currently supported through the DELTAS Africa Initiative grant # DEL-15-011 to THRiVE-2, from Wellcome Trust grant # 107742/Z/15/Z and the UK Government.

Author details

[a]Translational Research Laboratry, Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda. [b]Makerere University Lung Institute, College of Health Sciences, Makerere University, Kampala, Uganda. [c]Department of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda. [d]Mulago National Referral Hospital, Kampala, Uganda. [e]Respiratory and Infectious Diseases Unit, Kenyatta National Hospital, Nairobi, Kenya. [f]College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia. [g]Kenya Medical Research Institute, Nairobi, Kenya. [h]Department of Primary Care Respiratory Medicine, University of Groningen, Netherlands. [i]The National Aspergillosis Centre, Wythenshawe Hospital, The University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom. [j]Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom.

REFERENCES

1. Asher MI, Ellwood P. *The Global Asthma Report 2014*. Auckland: Global Asthma Network; 2014:16–17, 0473291266 0473291266.
2. Bateman ED, Hurd SS, Barnes PJ, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J*. 2008;31(1):143–178. https://doi.org/10.1183/09031936.00138707.
3. Wechsler ME. *Managing Asthma in Primary Care: Putting New Guideline Recommendations into Context*. Elsevier; 2009:707–717.
4. Adeloye D, Chan KY, Rudan I, Campbell H. An estimate of asthma prevalence in Africa: a systematic analysis. *Croat Med J*. 2013;54(6):519–531. https://doi.org/10.3325/cmj.2013.54.519.
5. Asher MI, Montefort S, Bjorksten B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases

One and Three repeat multicountry cross-sectional surveys. *Lancet*. 2006;368(9537):733–743. https://doi.org/10.1016/S0140-6736(06)69283-0.

6. Kwizera R, Musaazi J, Meya DB, et al. Burden of fungal asthma in Africa: a systematic review and meta-analysis. *PLoS One*. 2019;14(5). https://doi.org/10.1371/journal.pone.0216568. e0216568.
7. Zhong NS. New insights into risk factors of asthma. *Respirology*. 1996;1(3):159–166.
8. Johansson S, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: report of the nomenclature review committee of the world allergy organization, october 2003. *Journal of allergy and clinical immunology*. 2004;113(5):832–836.
9. Rasool R, Shera IA, Nissar S, et al. Role of skin prick test in allergic disorders: a prospective study in Kashmiri population in light of review. *Indian J Dermatol*. 2013;58(1):12–17. https://doi.org/10.4103/0019-5154.105276.
10. van Kampen V, de Blay F, Folletti I, et al. EAACI position paper: skin prick testing in the diagnosis of occupational type I allergies. *Allergy*. 2013;68(5):580–584. https://doi.org/10.1111/all.12120.
11. Heinzerling L, Mari A, Bergmann K-C, et al. The skin prick test–European standards. *Clinical and translational allergy*. 2013;3(1):3.
12. Ansotegui IJ, Melioli G, Canonica GW, et al. IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper. *World Allergy Organ J*. 2020;13(2):100080. https://doi.org/10.1016/j.waojou.2019.100080.
13. Bryant D, Burns M, Lazarus L. The correlation between skin tests, bronchial provocation tests and the serum level of IgE specific for common allergens in patients with asthma. *Clinical & Experimental Allergy*. 1975;5(2):145–157.
14. O'Brien RM. Skin prick testing and in vitro assays for allergic sensitivity. *Australian Prescriber*. 2002;25(4):91–93.
15. Haahtela T, Burbach GJ, Bachert C, et al. Clinical relevance is associated with allergen-specific wheal size in skin prick testing. *Clin Exp Allergy*. 2014;44(3):407–416. https://doi.org/10.1111/cea.12240.
16. Addo-Yobo EO, Custovic A, Taggart SC, Craven M, Bonnie B, Woodcock A. Risk factors for asthma in urban Ghana. *J Allergy Clin Immunol*. 2001;108(3):363–368. https://doi.org/10.1067/mai.2001.117464.
17. Motala C, Hawarden D. Diagnostic testing in allergy. *SAMJ: South African Medical Journal*. 2009;99(7):531–535.
18. Kirenga B. *African severe asthma Program (ASAP)*. February 28, 2017; 2017. Retrieved 14/08/2018, 2018, from https://clinicaltrials.gov/ct2/show/NCT03065920.
19. Kirenga B, Muttamba W, Mugenyi L, et al. *A Prospective Cohort Study of Severe Asthma and its Determinants in an African Population: The African Severe Asthma Program*. 25 BROADWAY, 18 FL, NEW YORK, NY 10004 USA: AMER THORACIC SOC; 2018.
20. Schatz M, Sorkness CA, Li JT, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. *Journal of Allergy and Clinical Immunology*. 2006;117(3):549–556.
21. Bousquet J, Mantzouranis E, Cruz AA, et al. Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. *J Allergy Clin Immunol*. 2010;126(5):926–938. https://doi.org/10.1016/j.jaci.2010.07.019.
22. Juniper EF, Svensson K, Mork AC, Stahl E. Modification of the asthma quality of life questionnaire (standardised) for patients 12 years and older. *Health Qual Life Outcomes*. 2005;3(1):58. https://doi.org/10.1186/1477-7525-3-58.
23. Dreborg S. The skin prick test in the diagnosis of atopic allergy. *J Am Acad Dermatol*. 1989;21(4 Pt 2):820–821. https://doi.org/10.1016/s0190-9622(89)70256-5.
24. ASCIA. *Skin prick testing for the diagnosis of allergic disease: a manual for practitioners*. March 2016; 2016. Retrieved 15 January 2020, from https://www.allergy.org.au/images/stories/pospapers/ASCIA_SPT_Manual_March_2016.pdf.
25. Heinzerling L, Mari A, Bergmann KC, et al. The skin prick test - European standards. *Clin Transl Allergy*. 2013;3(1):3. https://doi.org/10.1186/2045-7022-3-3.
26. Jensen-Jarolim E, Jensen AN, Canonica GW. Debates in allergy medicine: molecular allergy diagnosis with ISAC will replace screenings by skin prick test in the future. *World Allergy Organ J*. 2017;10(1):33. https://doi.org/10.1186/s40413-017-0162-3.
27. Malling HJ, Allesen-Holm P, Karved LS, Poulsen LK. Proficiency testing of skin prick testers as part of a quality assurance system. *Clin Transl Allergy*. 2016;6:36. https://doi.org/10.1186/s13601-016-0126-7.
28. Mpairwe H, Muhangi L, Ndibazza J, et al. Skin prick test reactivity to common allergens among women in Entebbe, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2008;102(4):367–373.
29. Liam CK, Loo KL, Wong CM, Lim KH, Lee TC. Skin prick test reactivity to common aeroallergens in asthmatic patients with and without rhinitis. *Respirology*. 2002;7(4):345–350.
30. Montealegre F, Quinones C, Michelen V, et al. Prevalence of skin reactions to aeroallergens in asthmatics of Puerto Rico. *P R Health Sci J*. 1997;16(4):359–367.
31. Koshak EA. Skin test reactivity to indoor allergens correlates with asthma severity in jeddah, Saudi Arabia. *Allergy Asthma Clin Immunol*. 2006;2(1):11–19. https://doi.org/10.1186/1710-1492-2-1-11.
32. Peat JK, Tovey E, Toelle BG, et al. House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med*. 1996;153(1):141–146. https://doi.org/10.1164/ajrccm.153.1.8542107.
33. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. *N Engl J Med*. 1990;323(8):502–507. https://doi.org/10.1056/NEJM199008233230802.
34. Arshad SH, Tariq SM, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. *Pediatrics*. 2001;108(2):E33.
35. Boulet LP, Turcotte H, Laprise C, et al. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clinical & Experimental Allergy*. 1997;27(1):52–59.

36. Arruda LK, Rizzo M, Chapman MD, et al. Exposure and sensitization to dust mite allergens among asthmatic children in Sao Paulo, Brazil. *Clinical & Experimental Allergy*. 1991;21(4):433–439.

37. Murray AB, Ferguson AC. Dust-free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. *Pediatrics*. 1983;71(3):418–422.

38. Woodcock A, Forster L, Matthews E, et al. Control of exposure to mite allergen and allergen-impermeable bed covers for adults with asthma. *N Engl J Med*. 2003;349(3):225–236. https://doi.org/10.1056/NEJMoa023175.

39. Peat J, Woolcock A. Sensitivity to common allergens: relation to respiratory symptoms and bronchial hyper-responsiveness in children from three different climatic areas of Australia. *Clinical & Experimental Allergy*. 1991;21(5):573–581.

40. Lau S, Illi S, Sommerfeld C, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. *Lancet*. 2000;356(9239):1392–1397. https://doi.org/10.1016/s0140-6736(00)02842-7.

41. Gøtzsche PC, Johansen HK. House dust mite control measures for asthma. *Cochrane Database of Systematic Reviews*. 2008;(2).

42. Andiappan AK, Puan KJ, Lee B, et al. Allergic airway diseases in a tropical urban environment are driven by dominant mono-specific sensitization against house dust mites. *Allergy*. 2014;69(4):501–509. https://doi.org/10.1111/all.12364.

43. Santos ABR, Chapman MD, Aalberse RC, et al. Cockroach allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. *Journal of Allergy and Clinical Immunology*. 1999;104(2):329–337.

44. Jeong KY, Kim CR, Park J, Han IS, Park JW, Yong TS. Identification of novel allergenic components from German cockroach fecal extract by a proteomic approach. *Int Arch Allergy Immunol*. 2013;161(4):315–324. https://doi.org/10.1159/000347034.

45. Arruda LK, Vailes LD, Ferriani VP, Santos ABR, Pomés A, Chapman MD. Cockroach allergens and asthma. *Journal of allergy and clinical immunology*. 2001;107(3):419–428.

46. Bush RK, Prochnau JJ. Alternaria-induced asthma. *J Allergy Clin Immunol*. 2004;113(2):227–234. https://doi.org/10.1016/j.jaci.2003.11.023.

47. O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC pulmonary medicine*. 2005;5(1):4.

48. Denning D, O'driscoll B, Hogaboam C, Bowyer P, Niven R. The link between fungi and severe asthma: a summary of the evidence. *European Respiratory Journal*. 2006;27(3):615–626.

49. Menzies D, Holmes L, McCumesky G, Prys-Picard C, Niven R. Aspergillus sensitization is associated with airflow limitation and bronchiectasis in severe asthma. *Allergy*. 2011;66(5):679–685.

50. Fairs A, Agbetile J, Hargadon B, et al. IgE sensitization to Aspergillus fumigatus is associated with reduced lung function in asthma. *American journal of respiratory and critical care medicine*. 2010;182(11):1362–1368.

51. Agarwal R, Noel V, Aggarwal AN, Gupta D, Chakrabarti A. Clinical significance of Aspergillus sensitisation in bronchial asthma. *Mycoses*. 2011;54(5):e531–e538. https://doi.org/10.1111/j.1439-0507.2010.01971.x.

52. Kwizera R, Bongomin F, Meya DB, Denning DW, Fahal AH, Lukande R. Mycetoma in Uganda: a neglected tropical disease. *PLOS Neglected Tropical Diseases*. 2020;14(4). https://doi.org/10.1371/journal.pntd.0008240. e0008240.

53. Kwizera R, Bongomin F, Lukande R. Deep fungal infections diagnosed by histology in Uganda: a 70-year retrospective study. *Med Mycol*. 2020:1–9. https://doi.org/10.1093/mmy/myaa018.

54. WHO. *Prevention and Control of Chronic Respiratory Diseases in Low and Middle-Income African Countries: A Preliminary Report*. Geneva: World Health Organization; 2003. WHO/MNC/CRA/02.2 WHO/MNC/CRA/02.2 22.

55. Olanrewaju FO, Ajayi LA, Lorromeke E, et al. Masculinity and men's health-seeking behaviour in Nigerian academia. *Cogent Social Sciences*. 2019;5(1):1682111. https://doi.org/10.1080/23311886.2019.1682111.

56. Yawn BP, Wollan P, Kurland M, Scanlon P. A longitudinal study of the prevalence of asthma in a community population of school-age children. *J Pediatr*. 2002;140(5):576–581. https://doi.org/10.1067/mpd.2002.123764.

57. Wright AL, Stern DA, Kauffmann F, Martinez FD. Factors influencing gender differences in the diagnosis and treatment of asthma in childhood: the Tucson Children's Respiratory Study. *Pediatr Pulmonol*. 2006;41(4):318–325. https://doi.org/10.1002/ppul.20373.

58. Radhakrishnan DK, Dell SD, Guttmann A, Shariff SZ, Liu K, To T. Trends in the age of diagnosis of childhood asthma. *J Allergy Clin Immunol*. 2014;134(5):1057–1062. https://doi.org/10.1016/j.jaci.2014.05.012. e1055.

59. Guilbert TW, Morgan WJ, Zeiger RS, et al. Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. *Journal of Allergy and Clinical Immunology*. 2004;114(6):1282–1287.

60. Haldar P, Pavord ID, Shaw DE, et al. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med*. 2008;178(3):218–224. https://doi.org/10.1164/rccm.200711-1754OC.

61. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med*. 2012;18(5):716–725. https://doi.org/10.1038/nm.2678.

62. Romanet-Manent S, Charpin D, Magnan A, Lanteaume A, Vervloet D, Group EC. Allergic vs nonallergic asthma: what makes the difference? *Allergy*. 2002;57(7):607–613.

63. Gilliland FD. Outdoor air pollution, genetic susceptibility, and asthma management: opportunities for intervention to reduce the burden of asthma. *Pediatrics*. 2009;123(Suppl 3):S168–S173. https://doi.org/10.1542/peds.2008-2233G.

64. Kemp S, deShazo RD. *Relationships between Rhinosinusitis and Asthma*. Waltham, MA: UpToDate: UpToDate; 2012.